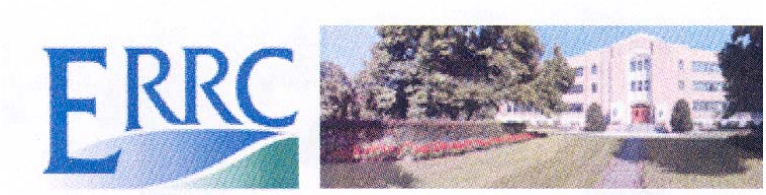

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Concerns with Minimal Processing in Apple, Citrus, and Vegetable Products

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CONTENTS

INTRODUCTION

Although the incidence of food-borne illnesses linked to fresh produce is low, there is increased awareness that fruits and vegetables can be contaminated with microbiological pathogens. For its microbiological sampling program of certain fresh fruits and vegetables, the U.S. Food and Drug Administration (FDA) conducted surveys of both imported and domestic produce. A 4% (44 of 1003 sampled) contamination rate was reported in published results for imported product (http://www.cfsan.fda.gov/~dms/prodsur6.html). In the interim report on domestic product, there was a 1.6% violation rate (http://www.cfsan.fda.gov/~dms/prodsur9.html). Two microbiological pathogens that can cause food-borne illnesses were present on the produce: *Salmonella* and *Shigella.*

With the shift in diet toward the consumption of more fresh fruits and vegetables and greater distribution distances from new geographic sources, there are more reported illnesses involve fresh produce (Tauxe et al., 1997). In the U.S. from 1988

to 1992, 64 outbreaks of disease were related to the consumption of fruits and vegetables resulting in 9 deaths (Bean et al., 1996). From 1993 to 1997, in 66 outbreaks with 2 deaths, fruits and vegetables were the vehicles of transmission (Olson et al., 2000). Because food-borne infections are sporadic and many go unreported, the exact number of cases related to produce is unknown (Tauxe et al., 1997). Due to changes in the food supply and food consumption patterns in the U.S., deaths caused by food-borne diseases are even more difficult to estimate (Mead et al., 1999). The food-borne outbreaks cause gastroenteritis and in severe cases, hospitalization is required. Bean et al. (1996) present guidelines listing syndrome, incubation time, and clinical identification procedures for the confirmation of gastrointestinal food-borne-disease outbreaks by etiological agent.

Beuchat (1996) and Sumner and Peters (1997) discussed and listed the microflora isolated from raw fruits and vegetables, including those involved in food-borne outbreaks. These authors listed the pathogens identified and the produce involved. The microbiology of minimally processed fresh fruits and vegetables was reviewed by Nguyen-The and Carlin (1994); these reviews discuss the human pathogens and spoilage microorganisms recovered and identified from fresh produce. Table 2.1 lists bacterial pathogens associated with fruits and vegetables.

In January of 1997, President Clinton announced a Food Safety Initiative, and a report by the U.S. Department of Health and Human Services, the U.S. Department of Agriculture, and the U.S. Environmental Protection Agency identified domestic fresh produce as an area of concern. In October of that same year, President Clinton initiated a plan entitled *Produce and Imported Food Safety Initiative* to assure safety of imported fruits and vegetables in the American diet. An alarming survey of imported produce in 1999, including broccoli, cantaloupe, celery, cilantro, loose-leaf lettuce products (radicchio, escarole, endive, chicory), parsley, scallions, and strawberries, found 40 out of 1000 samples tested positive for bacterial pathogens. Of this contaminated produce, 35 samples were contaminated with *Salmonella* and 9 with *Shigella*. No *Escherichia coli* O157:H7 was found (FDA, 2001).

Fruit and vegetable contamination problems can occur in the growing environment. During growth the fruit or vegetable can become contaminated from sources such as soil, animals, birds, and insects. Following production, the processes of harvesting, washing, cutting, slicing, packaging, and shipping can create additional conditions where contamination can occur. When produce is consumed in the raw, as is the case with fresh-cuts, harmful microorganisms may be present and ingested. In October of 1998, the Food and Drug Administration issued *Guidance for Industry — Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables,* which describes good agricultural and manufacturing practices (GAPs and GMPs, respectively). These guides cover water quality, manure management, worker training, field and facility sanitation, and transportation (FDA, 2001).

Traditionally, fresh fruits and vegetables were considered safe to eat raw — straight from the field, but now bacterial pathogens are being found on or in the fruit or vegetable. Today the consumer is advised through the news media to wash fresh fruits and vegetables before eating. Commercial washing kits are available to clean the raw produce. Consumers, particularly the young, elderly, or immunocompromised

TABLE 2.1
Bacterial Pathogens and Associated Fruit, Vegetable, and Juice Vehicles

Pathogen	Fruit/Vegetable/Juice	Ref.
Aeromonas spp.	Vegetables, sprouts	Escuder et al., 1999; NACMCF, 1999; Velázquez et al., 1998
Listeria monocytogenes	Cabbage, lettuce, salad	Beuchat, 1996; Kakiomenou et al., 1998; NACMCF, 1999; Nguyen-The and Carlin, 1994; Odumeru et al., 1997; Thompson and Powell, 2000
E. coli	Lettuce, melons, cantaloupe, cabbage, unpast. apple juice	Ackers et al., 1998; Beauchat, 1996; Castro-Rosas and Escartin, 2000; Cody et al., 1999; NACMCF, 1999; Nguyen-The and Carlin, 1994; Park and Beuchat, 1999; Tauxe et al., 1997
Salmonella spp.	Tomato, alfalfa sprout, salad, melon	Castro-Rosas and Escartin, 2000; Kakiomenou et al., 1998; Maxcy, 1978; NACMCF, 1999; Park and Beuchat, 1999; Thompson and Powell, 2000; Wei et al., 1995
Staphylococcus aureus	Salads, sprouts	Fowler and Foster, 1976; Maxcy, 1978; Thompson and Powell, 2000
Bacillus cereus	Sprouts	NACMCF, 1999; Thompson and Powell, 2000
Shigella	Onion, lettuce, cabbage	Beuchat, 1996; Escudero et al., 1999; Satchell et al., 1990
Vibrio cholerae	Alfalfa sprouts	Castro-Rosas and Escartin, 2000
Klebsiella	Dried bush okra, sprouts	Mpuchane and Gasha, 1996; NACMCF, 1999; Thompson and Powell, 2000
Campylobacter	Vegetables	Escudero et al., 1999; Park and Sanders, 1992
Pseudomonas	Vegetables	Nguyen-The and Carlin, 1994
Clostridium botulinum	Vegetables	Olsen et al., 2000
Yersinia enterliticus	Lettuce	NACMCF, 1999

for any reason, is now warned by government advisories that eating raw sprouts or drinking unpasteurized fruit drinks can make them ill (Tauxe et al., 1997).

Because of high water activity (a_w) and nutrient content, fresh produce can support the growth of a variety of disease-causing microorganisms (Sumner and Peters, 1997). Table 2.1 lists produce types from which the bacterial pathogens were isolated and identified. Some produce types (sprouts, lettuce, salad, and melons) were identified as vehicles of more than one pathogen species.

Today fresh fruits and vegetables can be purchased in a variety of forms. The produce can be whole, minimally processed (peeled) whole, cut into pieces, or in the form of unpasteurized juices. When whole, the produce is sold individually and usually not packaged. Wells and Butterfield (1999) have shown that pathogenic microorganisms such as *Salmonella* can grow in the wound area of a damaged, intact fruit or vegetable. When minimally processed, the produce can be packaged in pouches with and without modified atmosphere, in plastic clam packs, or in trays

covered with a polymeric wrap. The packaging is necessary because the fruit has lost all or part of its protective covering (peel). The cut and exposed surfaces of the minimally processed fruits or vegetables are a main concern because microorganisms can grow there (Nguyen-The and Carlin, 1994).

Another form is unpasteurized fresh juice processed in a food plant, in the store, or in the home. If a bacterial pathogen is present on or in the produce or on any processing surface, it will have an almost unlimited food source for growth in the unpasteurized juice, resulting in a food-borne outbreak (Cook et al., 1998, Steele et al., 1982). Pathogens can grow in apple and orange juices if the produce is not pasteurized before packaging, is temperature abused, or is kept for a long time (Cook et al., 1998; Doyle and Mazzota, 2000). Because food-borne illness involving young children has been traced to drinking untreated juices, the FDA has issued a consumer alert about the health risk of drinking untreated juices (http://www.cfsan.fda.gov/~dms/juicsaf2.html).

It was thought that the high acidity of some juices would inhibit the growth of pathogenic bacteria. In apple cider, which has a naturally low pH, *E. coli* O157:H7 was reported to survive and was acid tolerant in trypticase soy broth >pH 4 (Miller and Kasper, 1994). In 1996 an outbreak of *E. coli* O157:H7 infection from drinking unpasteurized commercial apple juice resulted in one death (Cody et al., 1999). *Salmonellae* are reported to survive in orange juice of pH 3.5 at refrigerated temperatures (Parish et al., 1997). *Salmonella* Hartford was associated with a food-borne outbreak linked to unpasteurized orange juice (Cook et al., 1998).

FRESH-CUT FRUITS AND VEGETABLES

High consumer demand for healthy foods has led to the exponential growth of the fresh-cut produce industry. The International Fresh-cut Produce Association (IFPA) defines fresh-cut products as fruits or vegetables that have been trimmed, peeled, or cut into 100% usable product that is bagged or prepackaged to offer consumers high nutrition, convenience, and flavor while still maintaining freshness (IFPA, 2001). Fresh-cuts are one category of minimally, or lightly, processed fruits and vegetables. These products should be in a raw state, not frozen or thermally processed, and ready to eat or cook (Anonymous, 1998a, b). Minimally processed products can also include unpasteurized, fresh juices.

The rapid growth of fresh-cuts in the U.S. over the past 10 years can be attributed to an increased consumer awareness of the nutritional benefits of fruits and vegetables, busy lifestyles, and spending power. Fresh-cut retail sales in the U.S. in 1994 were $5.8 billion (Hodge, 1995) and have reached more than 10% of the U.S. fresh vegetable and fruit market ($8.8 billion in 1998); sales are projected to increase to $19 billion by 2003 (Greenleaf, 1999). This value-added product has great potential for the fresh produce industry; however, several problems have limited commercial development, especially for fresh-cut fruits. Among the most serious are browning, softening, flavor deterioration, microbial decay, and safety. More specifically, browning, softening, and microbial decay were found to be limiting factors for the shelf-life of fresh-cut apples (Lakakul et al., 1999), pears (Gorny et al., 2000; Dong et al., 2000), peaches (Gorny et al., 1998), and kiwi fruit (Agar et al., 2000).

Maintenance of quality and control of microbial populations are problems for the fresh-cut produce industry. A combination of treatments is often necessary to maintain physiological quality and limit microbial growth. Solutions have included various chemical dips (Gorny et al., 1999; McHugh and Senesi, 2000; Dong et al., 2000), the use of certain cultivars and maturity levels that have superior stability (Gorny et al., 2000), controlled or modified atmospheres (Lakakul et al., 1999; Gorny et al., 1999), high-pressure processing (Boynton, 1999), and irradiation (Prakash et al., 2000).

New technologies, such as modified atmosphere packaging (MAP), have extended shelf-life and reduced decay and spoilage organisms, resulting in a shift in microbial population dynamics that may favor growth of human pathogens. In addition, when spoilage organisms are controlled, the food may look and smell edible even when it is not safe to eat. The change in atmosphere created by MAP, and fruit respiration (relative increase in CO_2 and decrease in O_2), may allow *Clostridium botulinum* to grow and form toxin, especially at elevated storage temperatures. Lower O_2 levels created by MAP reduce growth of aerobic spoilage organisms (Farber, 1991; Hotchkiss and Banco, 1992). Germination of *C. botulinum* spores is stimulated by elevated CO_2 (Enfors and Molin, 1978; Foegeding and Busta, 1983), as has been demonstrated with packaged, cut honeydew melons. Melons were inoculated with *C. botulinum*, then treated with UV light to inactivate vegetative organisms and packaged using passive MAP. This resulted in marginal spoilage and botulinal toxin formation (Larson and Johnson, 1999).

Fresh-cut produce deteriorates faster than its intact counterpart (Cantwell, 1995) due to the wounding associated with processing. This affects the stability of the produce as a result of biochemical and physiological changes (Brecht, 1995; Saltveit, 1997). Problems include changes in color (browning), flaccidity (loss of water), and microbial contamination at the cut surface (Brecht, 1995; King and Bolin, 1989; Varoquaux and Wiley, 1994). The surface structure of lettuce has been shown to protect *E. coli* O157:H7 cells or other pathogens from chlorine inactivation (Liao and Sapers, 2000; Takeuchi and Frank, 2001; Ukuku and Sapers, 2001). Wounding induces signals that elicit physiological and biochemical responses throughout the tissue (Ke and Saltveit, 1989; Saltveit, 1997). Wounding plant tissues makes them more susceptible to attack by plant pathogenic microorganisms and contamination with human pathogens.

The cut surface of any processed vegetable can support microbial growth. In addition, the cut vegetable continues to metabolize, producing more nutrients that become available for microbes. The natural microbiological counts of minimally processed produce including leaf and cut lettuce were reported by Beuchat (1996), Nguyen-The and Carlin (1994), and Sumner and Peters (1997). Aerobic mesophilic counts can range from 10^3 to 10^8/g, depending on the produce variety and geographic location (Beuchat, 1996). Recent studies (Escudero et al., 1999; Park and Beuchat, 1999; Velázquez et al., 1998; Wei et al., 1995) have documented the growth of inoculated pathogens (*Salmonella, Aeromonas, Yersinia, Vibrio. cholerae*) on produce; *E. coli* O157:H7 was confirmed as the agent in an outbreak involving leaf lettuce (Ackers et al., 1998). It has been shown (Kakiomenou et al., 1998; Jacxsens et al., 1999) that when minimally processed products are contaminated and stored

at 4 to 7°C, psychotropic bacterial pathogens can grow on the produce. Kakiomenou et al. (1998) demonstrated that when fresh-cut salad vegetables were packaged under modified atmosphere and stored at 4°C, *S. Enteritidis* and *Listeria monocytogenes* survived, and Jacxsens et al. (1999) showed that *L. monocytogenes* and *Aeromonas* spp. survived in fresh-cut salad vegetables stored at 7°C.

Bacteria account for the major portion of microflora on vegetable salads, but exact numbers vary according to time of year and geographic location (Garcia-Gimeno and Zurera-Cosano, 1997; Hagenmaier and Baker, 1998; King et al., 1991; Manvell and Ackland, 1986). Pathogens such as *L. monocytogenes* can also grow in contaminated produce at refrigerated temperatures (Odumeru et al., 1997). When the storage temperature of packaged salads was increased (abuse), lactic acid bacteria were the dominant species. Garcia-Gimeno and Zurera-Cosano (1997) suggested modeling the growth of lactic acid bacteria to predict the shelf-life of packaged salads; as a temperature and spoilage indicator, this model can be used to set the pull date of the product before the pathogens outgrow the nonpathogens.

Whole fresh fruits and vegetables with bacterial soft rot and fungal rot were shown to have a high incidence of contamination with *Salmonella* spp. (Wells and Butterfield, 1997, 1999). These areas of contamination become the inoculum for cross-contamination if the cutting utensil is not cleaned properly. Wells and Butterfield (1997) reported that contamination of wash water during rinsing of soft rotted fruit and vegetable could cause problems. Such contamination during washing can explain the increase of *Salmonella* and *E. coli* O157:H7 outbreaks associated with unpasteurized fresh juices (Cody et al., 1999; Cook et al., 1998).

If washing equipment becomes contaminated with the pathogen, a biofilm may develop. The biofilm structure can provide a protective environment for pathogens and reduce the effectiveness of any sanitizer or inhibitory agents used. The results of different sanitizers (free chlorine, acidified sodium chlorite, hydrogen peroxide, lactic acid, lactic acid plus chlorine and Tsunami [peroxyacetic acid]) as effective agents against *Salmonella, E. coli* O157:H7, *A. hydrophila,* and *Y. enterocolitica* on fresh produce were reviewed by Escudero et al. (1999), Park and Beuchat (1999), and Velázquez et al. (1998). Not one single agent was shown to be effective against all pathogens. Rajkowski and Thayer (2000) recently reported that the use of irradiation at a level of 2 kGy was effective in achieving a 5-log kill of *Salmonella* and *E. coli* O157:H7 on radish sprouts. Further investigation of the use and keeping quality of irradiated produce is necessary.

One developing technology is the use of antimicrobial edible packaging based on cellulosic ethers, fatty acids, and nisin. Nisin is an antimicrobial peptide that is effective against gram-positive bacteria (Coma et al., 2001).

MINIMALLY PROCESSED APPLES AND CITRUS

Cut apples are a nutritious and popular snack, ideal for airlines and schools and as a packaged product. Commercial products have appeared on the East and West Coasts (Anonymous, 1998c). New methods for washing whole apples prior to cutting include hot-water immersion (Fleischman et al., 2001), flatbed brush washing (Annous et al., 2001), and the use of sanitizers for reducing populations of *E. coli*

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(Sapers et al., 1999). These methods were effective only if the *E. coli* was not internalized. Hot-water immersion is effective in reducing or eliminating the microorganism if the inoculum is dropped onto the surface of the apple (Fleischman et al., 2001), whereas, if inoculated by submersion, brush washing and sanitizers can reduce only the surface population (Buchanan et al., 1999; Sapers et al., 1999; Annous et al., 2001).

However, browning and product safety are issues for the cut fruit. Browning can be inhibited by erythorbic or ascorbic treatments (Sapers et al., 1990; Sapers and Ziolkowski, 1987). Human pathogens and spoilage organisms are not so easily controlled. Fresh-cut apples were recalled due to possible *L. monocytogenes* contamination (SafetyAlerts.com, 2001); *L. monocytogenes* survived and increased on "Delicious" apple slices stored at 10 or 20°C in 0.5% O_2 and 15% CO_2 (Conway et al., 2000). When *L. monocytogenes* was inoculated into decayed apple tissue with the spoilage organism *Glomerella cingulata*, it increased in population, but it did not survive after 5 days in fruit infected with the spoilage organism *Penicillium expansum* (Conway et al., 2000). This may have been a pH effect because the pH increased in decaying tissue with *Glomerella cingulata* while, with *Penicillium expansum*, the pH decreased (Conway et al., 2000).

Total CFU/ml blended apple tissue on plate count agar was 4×10^3 for cut apple stored 2 weeks at 4°C without surface treatment and for 8 in. slices treated with a cellulose coating containing ascorbic acid, citric acid, and potassium sorbate (Baldwin et al., 1996). After 4 weeks of storage, the microbial populations on the fruit without surface treatment were 10^4, while treated fruit populations were 78 CFU/ml (Baldwin et al., 1996). Included in the storage atmospheres of natural apple volatiles, hexanal and trans-2-hexenal were shown to prolong the lag phases of inoculated yeast (*Pichia Subpelliculosa*) and reduced growth potential of naturally occurring bacteria, thus extending product shelf-life (Corbo et al., 2000). Hexanal vapor inhibited hyphae growth of fungi, *Pencillium expansum* and *Botrytis cinerea*, on apple slices (Song et al., 1996). The effect of these vapors on human pathogens is not known.

Like apple, peeled citrus segments, particularly orange and grapefruit, would be a popular and nutritious snack. The peel of citrus consists of a thin layer of colored flavedo and an inner layer of spongy white albedo tissue, which is difficult to remove from the fruit. Subsequent section production is labor intensive in that each section must be cut by hand from the peeled fruit. Traditionally, the fruits were steamed to loosen the peel, peeled by machine, and treated with lye. Cut sections were small and friable and necessitated liquid packing (Bruemmer et al., 1978).

The USDA Citrus and Subtropical Products Laboratory has developed a novel technology consisting of vacuum infusion of pectinases to facilitate removal of the peel from oranges and grapefruit (Bruemmer and Griffin, 1978; Bruemmer, 1981). This has resulted in fruit devoid of the adhering albedo, and sections that were easily separated. The mode of action of the process is the de-polymerization of middle lamellar pectins by the food-grade pectin-degrading enzymes, resulting in digested albedo and diminished segment membrane integrity. The resulting unpasteurized, dry-pack segments could provide a product with the quality of fresh fruit if flavor, texture, and microbial deterioration could be controlled (Baker and Bruemmer,

1989). This procedure and similar technologies have been commercialized in Florida (Stanley, 1996) and South Africa.

The vacuum infusion method requires that freshly washed orange or grapefruit be warmed to 30°C core temperature by immersion in warm water or holding overnight at 32°C. Fruit is then immersed in boiling water for 90 seconds to minimize contamination of segments by surface microflora, followed by scoring radically three times from stem to blossom end, which penetrates the flavedo and facilitates infusion of the enzyme solution. The technology is temporarily licensed to Pre-peeled Fruit Inc. in Groveland, Florida, and the pre-peeled product is marketed in Florida grocery stores (Stanley, 1996).

Other methods of peeling citrus have been developed. One patented by Adams and Kirk (1991) involves pressure rather than vacuum infusion of enzymes. Vacuum or pressure can be used to infuse water (without enzymes) into the pectin rich inner portion of the peel albedo (Pao et al., 1996). Infusion of water alone facilitates peeling and results in firm fruit with less juice leakage than with enzyme peeling (Pao et al., 1996), but a thin layer of albedo tissue and threads remains attached to the peel.

While these methods are promising, they each have inherent problems. One is microbial instability. Because the segments are not pasteurized, they contain indigenous microflora. To monitor microbial problems, grapefruit segments were stored in polyethylene bags, with or without 0.2% (w/v) potassium sorbate, at 2°C and examined periodically for yeast and bacteria. While bacterial contamination of segments was found to be low and declined during storage, initially low yeast contamination increased rapidly after 3 weeks of storage. This was controlled by 0.2% potassium sorbate dips (Baker and Bruemmer, 1989). Pao et al. (1998) challenged peeled sections with human pathogens including *E. coli* 0157:H7, *L. monocytogenes*, and *Staphylococcus aureus*. Refrigeration of inoculated sections reduced growth of all pathogens and caused population reduction of *Salmonella* spp. and *S. aureus*, but growth was observed with all pathogens at 24°C. Juice leakage may reduce the pH of contacted surface portions of the cut or peeled fruit. The reduction of surface pH inhibits some competitive microflora but favors the growth of spoilage yeasts and molds (Pao et al., 1997). Irradiation of fruit salad containing cantaloupe, pineapples, and orange sections using 0.5 kGy reduced the microbial populations by more than 90%, but populations subsequently increased during storage (Hagenmaier and Baker, 1997).

Unlike the fresh-cut or peeled products, unpasteurized juices from apple and citrus have been commercially available for many years and have a record of safety problems (Parish, 1997) (Table 2.2). Inoculated *Salmonellae* survived 27 days at pH 3.5, 46 days at pH 3.8, 60 days at pH 4.1, and 73 days at pH 4.4 in inoculated pasteurized orange juice (Parish et al., 1997). In 1996, nonpasteurized apple juice and juice blends from a processing facility in California were determined to be contaminated with *E. coli* 0157:H7 (CFDC, 1996). Freshly pressed, unpreserved apple cider can support *E. coli* O157:H7 organisms; the risk for this can be reduced by washing and brushing apples before pressing and by preserving the cider with sodium benzoate (Besser et al., 1993).

In 1995 a salmonellosis outbreak from orange juice implicated a citrus-processing facility and was linked to amphibians (Parish 1997; CFDC, 1995). *Salmonella* cells in the orange juice were associated with population levels of fecal coliforms and *E.*

TABLE 2.2
Disease Outbreaks from Consumption of Apple and Orange Juices

Year	Disease Vehicle	Causative Microorganism	Ref.
1923	Sweet cider	*Salmonella typhi*	Paquet, 1923
1994	Orange juice	*S. typhi*	Duncan et al., 1946
1962	Orange juice	Hepatitis A	Eisenstein et al., 1963
1966	Orange juice	Gastroenteritis agent	Tabershaw et al., 1967
1974	Apple cider	*S. Typhimurium*	Centers for Disease Control, 1967
1980	Apple cider	Enterotoxigenic *E. coli*	Steele et al., 1982
1989	Orange juice	*S. Typhi*	Birkhead et al., 1993
1991	Apple cider	*E. coli* 0157:H7	Besser et al., 1993
1992	Orange juice	Enterotoxigenic *E. coli*	Singh et al., 1995
1993	Apple cider	*Cryptosporidium*	Millard et al., 1994
1994	Orange juice	Gastroenteritis agent	FDA, 1994
1995	Orange juice	*S. hartford, S. gaminara, S. rubislaw*	Centers for Disease Control, 1995
1995	Orange juice	*E. coli*	Singh et al., 1995
1996	Apple juice	*E. coli* 0157:H7	Centers for Disease Control, 1996
1996	Apple juice	*E. coli* 0157:H7	Centers for Disease Control, 1997
1996	Apple juice	*Cryptosporidium parvum*	Centers for Disease Control, 1997

Source: Modified from Parish, M.E., *Crit. Rev. Microb.*, 23:109–119, 1997.

coli (Parish, 1998). Inoculation of *S. hartford* into refrigerated orange juice resulted in a maintained population level for 5, 10, 15, and 20 days at pH 3.5, 3.8, 4.1, and 4.4, respectively (Parish et al., 1997). Inactivation of *Lactobacillus plantarum* in orange–carrot juice was accomplished by means of high-intensity pulsed electric fields as a nonthermal preservation method (Rodrigo et al., 2001). Although nonthermal pasteurization of fruit juices has been demonstrated, it is not yet commercially practical. Isostatic high pressure, pulsed light, pulsed electric field, and filtration are several methods reported in the literature (Parish, 1997). A combined low-temperature, high-pressure treatment reduced counts of *E. coli* O157:H7 and various serovars of *Salmonella* in fruit juices. The pathogens were found to be most sensitive in grapefruit juice and least sensitive in apple juice at 615 MPa for 2 min at 15°C (Teo et al., 2001). UV light pasteurization is being tested on apple juice under simulated commercial conditions at a pilot plant at Illinois Institute of Technology's National Center for Food Safety and Technology in Sumit Argo, Illinois (Hollingsworth, 2001).

SPROUTS AND SPROUT SEEDS

Sprouts from a large variety of seeds (alfalfa, clover, broccoli, radish, and sunflower) are usually consumed raw, whereas mung and soy sprouts are usually cooked. The seeds are germinated and grown hydroponically in trays or drums; growth temperature will vary depending on the time of year and geographic location of the sprouting facility (Beuchat, 1996). The ambient temperature and moist environment are ideal

for raising sprouts, but they are also ideal for microbial growth. In the past decade, more than 12 reported outbreaks of sprout-related illness worldwide have been reviewed extensively (Beuchat, 1996; NACMCF, 1999a, b; Taormina et al., 1999; Tauxe et al., 1997; Thompson and Powell, 2000). Of the pathogens isolated, *Salmonella* spp. is the most common, followed by *E. coli* O157:H7 (27) and, more recently, *Klebsiella pneumoniae* (Thompson and Powell, 2000). As a result of these outbreaks, two recent reports were issued on the microbiological safety (NACMCF, 1999a, b) and the risk factors associated with consumption of fresh sprouts (Thompson and Powell, 2000). Of the different varieties of sprouts, alfalfa sprouts were found to have the largest number of outbreaks associated with their consumption. In 1999, the U.S. Department of Health and Human Services advised consumers in a press release of the risks associated with eating raw sprouts, especially alfalfa sprouts (U.S. Dept. Health and Human Services, 1999).

The pathogens found on or in sprouts (*Salmonella* spp. and *E. coli* O157:H7) originate from the seeds used for sprouting (NACMCF, 1999a, b; Thompson and Powell, 2000). In addition to these pathogens, Castro-Rosas and Estartín (2000) reported that alfalfa sprouts grown from *V. cholerae* O1-contaminated seeds were positive for the microorganism, even after treating the seeds with 100 mg/L of free chlorine from sodium hypochlorite.

Rice seeds are one of the other seeds being considered for sprouting because of the increased nutrient benefits. Piernas and Guiraud (1997) found that a treatment of 5 min at 60°C in a 1000-ppm solution of sodium hypochlorite resulted in up to a 5-log reduction in aerobic plate counts without affecting germination. There was no information whether this treatment would be effective against pathogens. To date, in the U.S. the only approved FDA guideline for the sprout industry is the use of 20,000 ppm of calcium hypochlorite solution, which still cannot guarantee a safe product. Dr. William Fett of the Agriculture Research Service (Wyndmoor, PA) stated through personal communication that the 20,000-ppm treatment with calcium hypochlorite solution does negatively affect the germination of some sprout seed varieties such as wheat. The other limiting factors are the disposal problems and safety of the workers.

EMERGING PATHOGENS

Three pathogens isolated from fruits and vegetables currently being studied are *Campylobacter, K. pneumoniae,* and *Shigella* spp. *C. jejuni* is the most common reported cause of food-borne infection in the U.S. (Altekruse et al., 1999) and is now more common than salmonellosis in England, Canada, and Australia (Park and Sanders, 1992). Two produce-related *C. jejuni* outbreaks occurred between 1973 and 1989 (Smith and Tamplin, 2000). In their sampling of fresh produce, Park and Sanders (1992) reported that *Campylobacter* isolates were found at rates of 1.6 to 3.3% in unwashed vegetables.

The other pathogen, *K. pneumoniae*, was believed to have contaminated alfalfa sprouts recalled in Canada in 1999 (Wargurton, 1999). Since this recall, Health Canada has developed a method for the isolation and identification of *K. pneumoniae* (Warburton, 1999).

Shigella spp. was isolated and identified from the domestic and imported fresh fruits and vegetables surveyed by the FDA (http://www.cfsan.fda.gov/~dms/prodsur9/htm and ~dms/prodsur6.htm).

CAUSES OF PRODUCE CONTAMINATION

The means by which fresh produce becomes contaminated with bacteria are outlined in reviews by Beuchat (1996) and Sumner and Peters (1997). Cross-contamination occurs during processing in the cutting or shredding operation (Garg et al., 1990). During this operation, proper cleaning is recommended (NACMCF, 1999b). There are concerns about the adequacy of some sanitation practices for fruit destined for unpasteurized juice or fresh-cut products because the raw fruit quality and degree of contamination affects the fresh-cut or juice product (Eleftheriadou et al., 1998). Sometimes the microflora on fruit from the field are not sufficiently removed in the packing and processing houses (Pao and Brown, 1998).

Microorganisms present on the peel contaminate the cut surface of fruits and vegetables after cutting. This can be surveyed, as was citrus in Florida (Pao and Brown, 1998). Citrus fruit surface microbial populations were monitored during washing and waxing in several commercial Florida packinghouses. The average total aerobic plate counts and yeast and fungal counts were 4.0 log CFU/cm^2 and 3.3 log CFU/cm^2, respectively, for incoming fruits and 2.1 log CFU/cm^2 and 1.3 log CFU/cm^2, respectively, after processing (washing and waxing). In this survey, no *Salmonellae* were found at any point and no *E. coli* was found after processing. When *E. coli* was applied to the fruit prior to processing at 4.8 log CFU/cm^2, the levels were reduced to 1.4 log CFU/cm^2 after washing and waxing (Pao and Brown, 1998).

Sapers et al. (1999) investigated better ways to sanitize apples contaminated with *E. coli*. They compared commercial washing formulations with 200 ppm Cl_2 and 5% H_2O_2 or combinations of H_2O_2 and commercial detergent formulations, both heated at 50°C or unheated and applied to apples inoculated with nonpathogenic *E. coli*. Heated commercial formulations resulted in a 2.5-log reduction in *E. coli* populations, 200-ppm Cl_2 resulted in a 2-log reduction, and heated H_2O_2 combined with surfactants resulted in a 3- to 4-log reduction in *E. coli* load.

Soil and water present on the surface of produce can support the growth and survival of pathogens. *L. monocytogenes* was detected in soil and vegetation and isolated from sewage sludge cake, commonly used as agricultural fertilizer in Iraq (Al-Gahazali and Al-Azawi, 1990). Tauxe et al. (1997) suggested that the increased use of manure instead of chemical fertilizers, especially in less developed countries, could introduce food-borne pathogens into the soil if the manure is not treated properly. Bryan (1977) reviewed those illnesses associated with contaminated foods by wastewater. The microbes listed in Table 2.1 are also listed in Bryan's review (1977). Rajkowski and Rice (1999) reported that when the coliform growth response (a microbial assay of water quality) of wastewater was greater than 2, *E. coli* O157:H7, *Salmonella* spp., and *V. cholerae* spp. could survive and grow.

There is also concern that farm workers harvesting produce from fields irrigated with wastewater can be a source of cross-contamination. A study by Ait Melloul and Hassani (1999) observed farm workers irrigating many vegetables, some of

which were consumed raw. The children of these farm workers had a higher incidence of salmonellosis than those children in other geographic areas. The affected children were observed playing in the field where the untreated wastewater was used as irrigation. The families of these farm workers also ate raw vegetables harvested from these fields.

With increased transportation and demand for fresh fruits and vegetables, the produce of such irrigated fields may be the source of future food-borne outbreaks. In addition to wastewater, river water is reported to support the growth of pathogens (Rajkowski and Rice, 1999).

Robinson and Adams (1978) studied the effect of ultraviolet treatment on contaminated irrigation water used to irrigate a celery crop. Their results showed that the u-v treatment was effective in cleaning up the polluted water supply. If polluted waters were used at any point in the fruit or vegetable operation, the produce, too, became contaminated (Ait Melloul and Hassani, 1999; Beuchat, 1996; Castro-Rosas and Escartin, 2000). Proper disinfecting of water used in produce operations is essential and, when economically feasible, sanitizing agents such as chlorine should be used. As with any water used on fresh produce, the microbiological quality of the irrigation water should be monitored.

SOLUTION

Each step in processing affects the microflora of fresh-cut vegetables (Garg et al., 1990), as does postprocessing handling and packing (Zagory, 1999). Identifying each point in the process and the possible ways contamination can occur is essential. The citrus packing line study is such an example (Pao and Brown, 1998). The National Advisory Committee on Microbiological Criteria for Foods (NACMCF, 1999b) has recommended several steps to assure microbiological safety of fresh produce and has developed seven specific recommendations. They are (1) good agricultural practices, (2) good manufacturing practices, (3) hazard analysis critical control point programs (HACCP), (4) training, (5) risk assessment, (6) research, and (7) better produce identification–tracing systems to investigate produce-related outbreaks. HACCP was considered the best guideline to assure produce safety. Kvenberg et al. (2000) developed a generic HACCP plan mandated for production of fruit and vegetable juices. In addition to HACCP, in the processing of fresh fruit and vegetables, guidance to ensure the microbiological safety of the produce for small catering operations worldwide was outlined by Mossel et al. (1999). Their guidelines parallel those recommended by NACMCF and their endeavor was to provide an unconditionally safer food supply.

FUTURE

With the proposed guidelines in place, there are still areas of research needed to assure a safe produce supply. Doyle and Mazzotta (2000) reviewed studies on the heat resistance of *Salmonellae* and suggested that research on the thermal resistance of *Salmonella* in fruit juices is needed. Research is also needed to determine the

radiation resistance of the pathogens on seeds used for sprouts and other fresh-cut fruits and vegetables. As mentioned previously, not one sanitizer is effective at the same exposure time, temperature, and strength for all possible pathogens and products. Research is needed to find one universal sanitizer that would be effective for all edible fruit and vegetable products, resulting in safer produce supply.

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